Anti-sialyl-di-lewis^a CAR T cells for effective anti-tumour therapy

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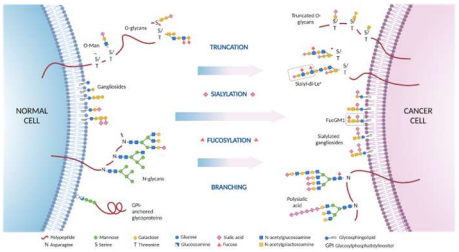
INTRODUCTION

- Targeting cancer-associated glycans can provide new targets for immunotherapy.
- Tumour cells show altered glycan expression that can be exploited to differentiate between cancer and self, but this requires the use of highly specific anti-glycan antibodies.
- We have an antibody (FG129) which targets sialyl-di-Lewis^a which is overexpressed on many cancer types including pancreatic, colorectal, gastric, ovarian, and lung.
- Chimeric antigen receptor (CAR) T cell therapy has the potential to target tumours with all the advantage of an antigen-specific T cell response, but without the dependence on MHC-presentation.
- Here we have engineered third generation anti-sialyl-di-lewis^a CAR T cells to target tumour cells.



Cancer associated glycans. Alterations to glycan via truncation, sialylation, fucosylation and branching can lead to altered glycan profiles on tumour cells. Anti-glycan antibodies with excellent specificity, bind strongly to tumours and show restricted normal tissue expression

Detection of sialyl-di-lewis^a on cancer tissues

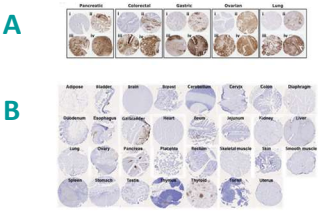


Figure 1. Strong differential tumour (A) versus normal human tissue (B) distribution via IHC of the FG129. Binding of FG129 by IHC to pancreatic, colorectal, gastric, ovarian, and lung TMAs (A). Representative images of different staining levels are shown: (i) negative, (ii) weak, (iii) moderate, and (iv) strong (magnification x20). Normal human tissue (AMSBIO) binding of FG129, showing limited binding in esophagus, gallbladder, ileum, liver, pancreas, and thyroid (magnification 20) (B).

Reference: Monoclonal Antibody Targeting Sialyl-di-Lewis^a–Containing Internalizing and Noninternalizing Glycoproteins with Cancer Immunotherapy Development Potential. Tivadar *et al.*, Mol Cancer Ther (2020) 19 (3): 790–801. https://doi.org/10.1158/1535-7163.MCT-19-0221

- Tumour microarrays show increased expression of sialyl-di-lewis^a compared to normal tissue
- Human tumour cell lines show expression of sialyl-di-lewis^a
  - High expression for COLO205 and BxPC3 cell lines
  - Low expression for HT-29 cell line
  - No expression on AGS cell line

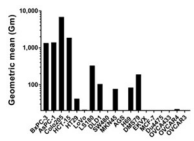


Figure 2. Overview of FG129 binding (33.3 nmol/L) to a range of pancreatic, colorectal, gastric, lung, and breast cancer cell lines via indirect immunofluorescence staining and flow cytometric analysis.

Engineering an anti-sialyl-di-lewis^a CAR T cell

- FG129 single chain fragment variable (scFv) were tested for antigen binding.
- Third generation CAR T constructs containing the antigen recognizing extracellular domain connected to human CD28 transmembrane region and intracellular domains consisting of the costimulatory human CD28 and 4-11B domains and a CD3ζ signalling domain were designed in a heavy light (HL) or light heavy (LH) orientation using 129scFv.

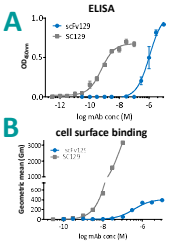


Figure 3. ELISA assays (A) and cell surface binding (B) were used to confirm binding of the FG129 single chain Fv (Sc-Fv) portions compared to parental SC129. ELISA (C) was also used to determine the binding of humanised h129 antibody and scFv in the LH or HL construct orientations using plate bound sialyl-di-lewis^a

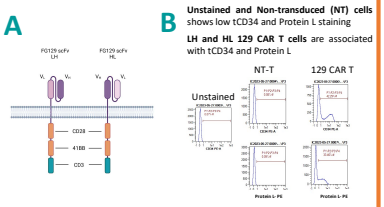


Figure 4. Schematic of CAR constructs (A) and staining of lentiviral particles expressing 129 CAR construct for transduction of human T cells (B). Transduction efficiency is measured through staining with protein L or for expression of marker tCD34 by flow cytometry.

In vitro assays show tumour recognition and killing

- Lentiviral transduced sialyl-di-lewis^a HL and LH CAR T cells recognise and kill sialyl-di-lewis^a expressing tumour lines
- Transduced CAR T cells kill high sialyl-di-lewis^a expressing Colo205, BxPC3 and moderate expressing HT29 cells but fail to kill low expressing/negative AGS cells *in vitro*

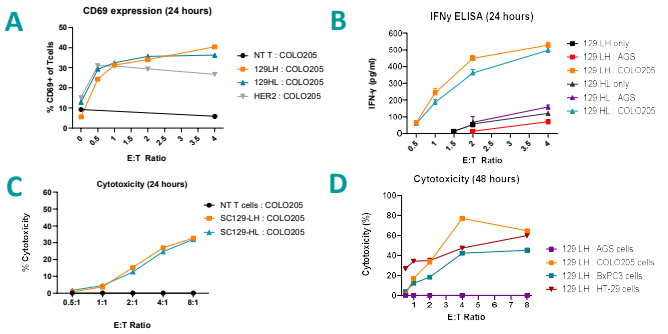


Figure 5.
The effect of FG129 CAR T cells were then assessed *in vitro*. FG129 CAR T cells in the LH (129 LH) or HL (129 HL) orientations were incubated with tumour cell lines that express high (COLO205), moderate (BxPC3), low (HT-29) or lack (AGS) sialyl-di-lewis^a. Non-Transduced (NT) T cells or 129 CAR T cells incubated without tumour cells were used as negative controls. T cells transduced with a commercially available HER2 construct were used as a positive control. Effects were assessed at different effector to target ratios (E:T). After 24 hrs the FG129 CAR T cell were stained for the activation marker CD69 (A) and IFNγ release was assessed by ELISA (B). Tumour cell killing was assessed by LDH assay after 24 hours (C) or 48 hours (D).

Anti-sialyl-di-lewis^a CAR T cells mediate a strong anti-tumour effect

- NSG mice implanted with Colo205 cells followed by injection of sialyl-di-lewis^a CAR T cells
- Sialyl-di-lewis^a CAR T cells destroy sialyl-di-lewis^a expressing Colo205 *in vivo*

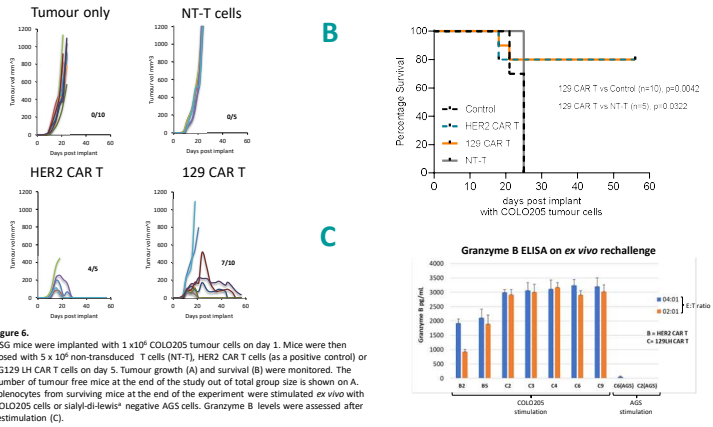


Figure 6.
NSG mice were implanted with 1 x10^6 COLO205 tumour cells on day 1. Mice were then dosed with 5 x 10^6 non-transduced T cells (NT-T), HER2 CAR T cells (as a positive control) or FG129 LH CAR T cells on day 5. Tumour growth (A) and survival (B) were monitored. The number of tumour free mice at the end of the study out of total group size is shown on A. Splenocytes from surviving mice at the end of the experiment were stimulated *ex vivo* with COLO205 cells or sialyl-di-lewis^a negative AGS cells. Granzyme B levels were assessed after restimulation (C).

CONCLUSIONS

- **Sialyl-di-lewis^a is expressed on the surface of many cancer cell types**
- **CAR T cells can be engineered to recognised sialyl-di-lewis^a using the 129 antibody previously characterised**
- **129 CAR T cells are activated by sialyl-di-lewis^a expressing cancer cell lines**
- **129 CAR T cells are associated with a strong anti-tumour effect *in vivo* in NSG mice**

Anti-sialyl-di-lewis^a CAR T cells can induce a strong anti-tumour response